

# EUROPEAN PATENT OFFICE

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TITLE : MODIFIED ENZYME HAVING  
CHOLESTEROL OXIDASE ACTIVITY

Met Thr Ala Gln Gln His Leu Ser Arg Arg Arg Met Leu Gly Met Ala  
1 5 10 15  
Ala Phe Gly Ala Ala Ala Leu Ala Gly Gly Thr Thr Ile Ala Ala Pro  
20 25 30  
Arg Ala Ala Ala Ala Lys Ser Ala Ala Asp Asn Gly Gly Tyr Val  
35 40 45  
Pro Ala Val Val Ile Gly Thr Gly Tyr Gly Ala Ala Val Ser Ala Leu  
50 55 60

Phe Cys Tyr His Pro Leu Gly Gly Cys Val Leu Gly Lys Ala Thr Asp  
485 490 495  
Asp Tyr Gly Arg Val Ala Gly Tyr Lys Asn Leu Tyr Val Thr Asp Gly  
500 505 510  
Ser Leu Ile Pro Gly Ser Val Gly Val Asn Pro Phe Val Thr Ile Thr  
515 520 525  
Ala Leu Ala Glu Arg Asn Val Glu Arg Ile Ile Lys Gln Asp Val Thr  
530 535 540  
Ala Ser  
545

ABSTRACT : PURPOSE: To obtain a modified enzyme-coding gene, having high stability, enabling the practice of measurement of cholesterol activities with high reliability, useful as a reagent, etc., for clinical tests and improved in thermostability and useful for creating the subject enzyme according to the genetic engineering.

CONSTITUTION: This modified enzyme-coding gene codes for an amino acid sequence in which at least one amino acid residue of an amino acid sequence ranging from the amino terminal to an active site is substituted, inserted or deficient in a primary structure of a precursor protein of a cholesterol oxidase (e.g. the primary structure having an amino acid sequence of the formula in the precursor protein of the cholesterol oxidase produced by Streptomyces sp. ASA-COO). A gene, etc., coding for the amino acid sequence in which Ser residue at the 103rd position is substituted with Thr residue are preferred. This creation of the modified enzyme can be achieved according to a genetic engineering technique such as transduction of a site-specific variation into the amino acid sequence of the formula.

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